IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Hillen *et al.*	)	Group Art Unit: 1636
Serial No.: 10/594,262	)	
Filed: July 9, 2007	)	Examiner: Qian, Celine X.

For PEPTIDE-BASED METHOD FOR MONITORING GENE EXPRESSION IN A HOST CELL

Declaration Of Dr. Andreas Burkovski

I, Dr. Andreas Burkovski, being of legal age, do hereby declare that:

1. My Curriculum Vitae is included as **Exhibit 1**.

2. I was provided with a copy of the following materials: (a) Office Action April 28, 2010; (b) Amendment and Response to Office Action of April 28, 2010; (c) Final Office Action July 6, 2011; (d) Submission in response to Final Office Action of July 6, 2011; (e) Toby, 2001, Methods, 24:201-217 ("Toby"); and (f) Manfredi et al., U.S. Patent 6,828,112 ("Manfredi"). I have read these documents and disagree with contentions of the Examiner for the reasons stated below:

3. I note that the Examiner has maintained rejection of pending claims 1, 4-8 in the July 6, 2011 Office Action as follows:

(i) Toby anticipates claims 1, 4, 5, 7 and 8 under 35 U.S.C. § 102(b). See pages 2-6 of the Office Action.

(ii) Toby and Manfredi render claim 6 obvious under 35 U.S.C. § 103(a). See pages 6-8 of the Office Action.

I will discuss each of these rejections and my opinions further below.

4. Toby does not anticipate claims 1, 4, 5, 7 and 8.

5. I understand that a claim is anticipated if all of the elements, features or "limitations" of the claim are found expressly or inherently in a single prior art document. The prior art reference must also enable the claimed invention.

6. The rejected claims, require, among other things, "modulating the activity of a regulatory biomolecule," specifically that "tag comprises the interacting residues of the interaction partner," and "binds to and modulates activity of said regulatory

biomolecule," and "assessing the expression level of the gene encoding the (poly)peptide of step (a)(ii) via a readout system."

7. In general, from the detailed description of the invention, I note that the invention relates to a method that determines the expression level of a gene. In this method, a peptide that can modulate a regulatory biomolecule such as TetR, is used as a tag. The tag is fused to a protein of interest. The tag binds and modulates the activity of the regulatory biomolecule. The nucleic acid binding activity of the biomolecule is thus regulated, and its active state is switched to a less active or inactive state. This switch in activity of the regulatory biomolecule following the binding of the tag to it causes the expression of a reporter gene located downstream of the binding site in the nucleic acid. For instance, a repressor (e.g., TetR) might be switched to its inactive state which will allow transcription of the gene controlled by the repressor. The expression level of the reporter gene indicates the amount of the tag and thereby the amount of the tagged protein. Stated otherwise, the expression level of the reporter gene will reflect the expression level of the tagged protein, and the amount of the tagged protein in the cell determines the readout obtained with the reporter system.

8. I note that the Examiner has taken the position that all of the limitations of claim 1 and other rejected claims are taught by or are necessarily present in the Toby reference. I respectfully disagree. Toby is about yeast two-hybrid method. That method is used to detect DNA binding domain/bait and transcription activation domain/prey (protein-protein) interactions. In yeast two-hybrid method, the bait must bind to regulatory sequences for transcriptional activation of a reporter gene. Thus, according to the yeast-two-hybrid system of Toby, the strength of the interaction between bait and prey defines the readout obtained with the reporter system (lacZ). In such a system, neither the bait nor the prey can activate transcription by itself. See also Toby Fig. 1 generally and the relevant text for details.

9. Toby is about yeast two-hybrid method. That method is used to detect DNA binding domain/bait and transcription activation domain/prey (or protein-protein) interactions. Toby teaches that the bait must remain bound to its recognition

sequence in the DNA whether the DNA is transcriptionally repressed or active. The interaction between bait that is bound to the DNA and prey causes the transcriptional activation of the reporter gene (e.g., lacZ) but that interaction must be such that it must not affect the nucleic acid binding activity of the bait. Thus, in Toby, the bait protein remains bound to the DNA before, during and after interaction with the prey protein. See Toby generally including Fig. 1. The prey protein does not regulate the nucleic acid binding activity of the bait. In fact, it is this absolute lack of any modulation in the bait's activity that is not only just essential for, but also the fundamental basis of the functionality of the yeast two-hybrid system described in Toby. The interacting protein's (prey's) tethering to the unmodulated/unaltered bait protein that that is bound to the DNA leads to the activation of reporter gene expression.

10. In the present invention, in contrast, the interacting tag binds to the regulatory biomolecule (e.g. Tet repressor) and causes the modulation of the biomolecule's affinity to its recognition sequence on the DNA. Essentially, the binding interferes with the affinity of the regulatory biomolecule for its recognition sequence on the DNA. This regulation of the nucleic acid binding activity of the biomolecule is a fundamental aspect necessary for the functionality of the claimed invention. The claimed invention does not concern two hybrid based approaches to study protein-protein interactions.

11. The yeast two-hybrid systems disclosed in Toby require, among other things, constant binding of the bait to the DNA to allow reporter gene readout. The method claimed in the present application requires the exact opposite, viz., the regulation of the nucleic aid binding activity of the bound bait protein in order to allow reporter gene readout.

12. The language pertinent to this contrasting feature is set forth, for example, in claim 1(a)(ii), viz., "said tag comprises the interacting residues of the interaction partner," and "binds to and modulates activity of said regulatory molecule." In the context of the invention described in the above application, the tag is a short peptide that interacts with the regulatory biomolecule (e.g., TetR protein), and modulates its activity. The tag is the constant part of the

interaction protein, the variable part being the cDNA/coding sequence of any gene of interest in the genome of an organism. In Toby, the interaction with the biomolecule occurs via the bound variable part of the interaction protein, and not via the tag. In the claimed method, the tag is specifically designed (to contain interacting residues) to interact with a regulatory biomolecule, e.g. TetR. It can be added to the coding sequence of any protein to detect the protein expression. Expression of this tagged protein is mediated by the endogenous genetic locus of the gene that was tagged. There is no such tag in Toby. Thus, Toby does not disclose the limitations set forth in claim 1(a)(ii). The "HA tag" (hemagglutinin epitope tag) referred to by the Examiner in the Final Office Action at pages 3 and 5, is also a constant part of the molecule described in Toby but this element does not mediate an interaction with the bait protein. The HA tag is one of the known epitope tags for antibody binding. Epitope tags are usually added to proteins which an investigator wants to visualize. Visualization can take place in a gel or a western blot, for example. In Toby, the HA tag is there to allow one to detect the expression of the interaction proteins (preys) by Western blotting with antihemagglutinin antibody. It is not there to bind to and modulate activity of a regulatory biomolecule or regulate the expression of the reporter protein located downstream of the recognition sequence of the regulatory biomolecule. These differences clearly represent material differences between the method claimed in the above application and Toby's system.

13. Another difference between Toby and the claimed invention is in that the limitations set forth in claim 1(b): "assessing the expression level of the gene encoding the (poly)peptide of step (a)(ii) via a readout system, wherein the readout system is provided by the nucleic acid molecule encoding a reporter protein" are not found, either expressly or inherently described, in the Toby reference. In this regard, I note the Examiner's assertion at page 3 of the Final Office Action that "the reporter readout reflects the expression [a qualitative feature] of the interacting protein (the prey)." I respectfully disagree with this assertion and refer to text in the paragraph bridging left and right columns at page

208 of Toby, which states that:

> ". . . the expression of the prey in addition to that of the bait should also be assayed before proceeding to the interaction test. ... Expression of the preys can be assessed by Western blotting with antihaemagglutinin antibody to detect preys expressed in pJG4-5."

This shows that Toby does not consider LacZ activity as a method to monitor the expression level of the prey. Rather, Toby expressly teaches the use of Western blotting to detect preys. Toby does not teach the use of a reporter protein to detect preys. In the type of systems disclosed in Toby, the interaction between bait and prey is variable, and the strength of that interaction is assessed. This is reinforced in the following sentence in the paragraph under "Overview" beginning at page 204:

> "Interaction between bait and prey is assessed both by the transcriptional activation of a colorimetric reporter, *lacZ* . . . and by positive growth selection using an auxotrophic reporter gene (*LEU2*)"

and the last sentence of the paragraph bridging left and right columns at page 208:

> "If quantitative ranking of the interactions is required, this can be accomplished by performing a liquid ONPG cleavage assay to measure β-galactosidase activity . . ."

Thus, the LacZ readout assay is used in Toby to quantify the strength of an interaction between bait and prey (regardless of different baits and preys or of different preys to a single bait), but not at all to assess the expression level of the prey protein as required by claim 1(b). See also Toby at page 216, last paragraph, where it teaches:

> "In conclusion, although powerful, the two hybrid system is only one of a battery of different techniques that allow detection and refined measurement of protein-protein interactions . . ."

Stated otherwise, I do not consider Toby as necessarily teaching the concept of using reporter readout to assess the expression level of the prey. Accordingly, Toby fails to teach a number of limitations of claim 1.

14. To anticipate, the Toby reference must also enable the claimed method, i.e., the reference must also show that one of ordinary skill in the art would know how to practice or to carry out the claimed method in light of the teachings therein. In my view, the Toby reference is inoperative and nonenabling with respect to the claimed method. Toby focuses exclusively on yeast two-hybrid systems. From the description of such systems, I cannot see any disclosure that necessarily teaches anything about the claimed method for monitoring the expression level of a gene in a host cell by modulating the activity of a regulatory biomolecule. Neither I nor others similarly skilled in the art would know how to practice or carry out the claimed method in light of the Toby reference. For example, Toby contains no guidance or examples as to how to design a tag molecule having interacting residues of an interaction partner of a regulatory biomolecule such that the tag binds to and modulates activity of the regulatory molecule. Further, for example, Toby contains no guidance as to how to assess the expression level of the gene encoding the interaction partner via a readout system, wherein the readout system is provided by the nucleic acid molecule encoding a reporter protein. These teachings are not inherently present in Toby. The present application describes the claimed method in detail including actual examples of the experimental procedure related to, for example, design of a tag molecule having interacting residues of an interaction partner of a regulatory biomolecule such that the tag binds to and modulates activity of the regulatory molecule. This sort of enabling information, absent in Toby, was not known as of July 6, 2001. Without that prior knowledge, arriving at the claimed method with a reasonably expectation of success will require undue experimentation even by one highly skilled in the relevant art. Therefore, in my opinion, Toby does not anticipate the rejected claims.

15. I understand that the following are considered in determining whether a patent claim recites obvious subject matter: (i) the scope and content of the prior art; (ii) the level of ordinary skill in the relevant art at issue; and (iii) the differences between the claimed invention and the prior art. If the differences between the claimed subject matter and the prior art are such that the claimed subject matter as

a whole would have been obvious at the time the invention was made to a person of ordinary skill in the art, then the claim is said to be obvious under 35 U.S.C. § 103(a).

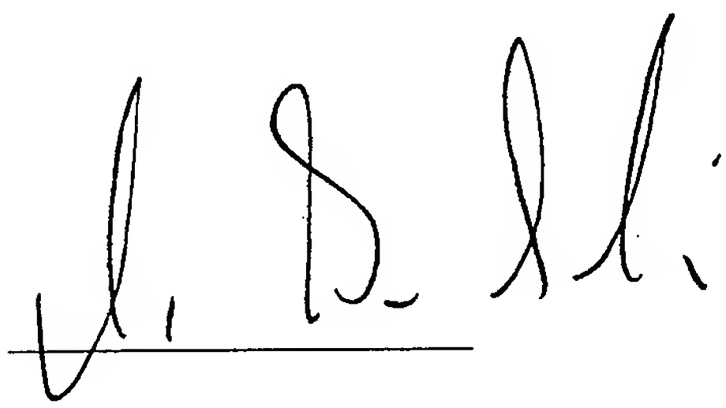
16. The combination of Toby and Manfredi does not render claim 6 obvious.

17. Claim 6 depends from or refers back to claim 1. I understand that claims in dependent form shall be construed to include all the limitations of the claim to which it refers.

18. Toby does not teach or suggest the invention in claim 1 as a whole. As I discussed above, Toby is about yeast two-hybrid systems. Like Tobi, Manfredi discloses yeast-two-hybrid systems. The two-hybrid systems of Toby and Manfredi require constant binding of the bait to the DNA without any modulation of the baits binding activity. Toby and Manfredi are not about monitoring the expression level of a gene in a host cell by modulating the activity of a regulatory biomolecule as set forth in the instant claims. Toby's teachings about yeast two-hybrid systems do not even remotely suggest, either explicitly or implicitly, the features required by the claimed invention. Manfredi does not fill the gaps in Toby. Manfredi does not teach or suggest anything about a tag molecule having interacting residues of an interaction partner of a regulatory biomolecule such that the tag binds to and modulates activity of the regulatory molecule. Manfredi does not suggest about assessing the expression level of the gene encoding the interaction partner via a readout system, wherein the readout system is provided by a reporter protein that confers resistance to an antibiotic. Neither Toby nor Manfredi represents a predictable solution to the problem of monitoring the expression level of a gene in a host cell by modulating the activity of a regulatory biomolecule. A skilled artisan would not have had a reasonable expectation of successfully arriving at the claimed method for monitoring the expression level of a gene in a host cell by modulating the activity of a regulatory biomolecule. Therefore, in my opinion, Toby and Manfredi do not render claim 6 obvious.

19. I make the statements in paragraphs 7-24 above based on my education, training and experience specified in **Exhibit 1.**

By signing below, I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true; and further that these statements were made with the knowledge that willful statements and the like so made are punishable by fine or by imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the application, any patent issuing there upon, or any patent to which this verified statement is directed.



Name: Prof. Dr. Andreas Burkovski

Date: 19. Dezember 2011

EXHIBIT 1

Andreas Burkovski, Prof. Dr. rer. nat., geb. 05.04.1963

Education

1983-1988	Study of Biology, University Osnabrück, Diploma in Biology 1988
	Supervisor of Diploma thesis: Prof. Dr. K. Altendorf
1988-1993	PhD research, University Osnabrück, PhD 1993
	Supervisor of PhD thesis: Prof. Dr. K. Altendorf

Academic and Research Appointments

1993-1994	Post-doctoral scientist, University Osnabrück
1994-1997	Post-doctoral scientist, Institute of Biotechnology, Research Centre Jülich
1997-2005	Group leader, Institute of Biochemistry, University of Cologne
2002	"Habilitation" in Biochemistry at the University of Cologne
	Mentor: Prof. Dr. R. Krämer
2005-present	Microbiology Professor (W2), Dept. of Biology, Chair of Microbiology, Friedrich-Alexander University Erlangen-Nuremberg

Honours and Awards

Fellowships of the *Studienstiftung des Deutschen Volkes* for Biology study and Ph.D. project.

Extramural Appointments

2000 - 2004	Coordinator of BMBF-Verbundprojekt "Neue Methoden zur Proteomanalyse: Anwendung und Verknüpfung mit Metabolomanalysen am Beispiel von *Corynebacterium glutamicum*".

Research interests and achievements

Research of the group focuses on physiology and biotechnology of mycolic acids-containing actinomyetes, including studies within the following areas:

- Uptake and metabolism of nitrogen sources in corynebacteria
- Nitrogen regulatory networks in corynebacteria and mycobacteria, including transcriptional and posttranslational control mechanisms
- Influence of nitrogen assimilation on amino acid production
- Integration of -omics technologies (transcriptomics, proteomics, metabolomics) to understand interaction of nitrogen and carbon metabolism in *C. glutamicum*
- Modelling of metabolic and regulatory networks
- Host-pathogen-interaction of corynebacteria.

Typically, in all projects a combination of various biochemistry and molecular biology tools including different global analysis techniques is applied in order to understand the physiology and regulation of the studied phenomena/microorganisms on different cellular levels.